

CLAIMS

1. A method for production and purification of a soluble heterologous fusion protein comprising a cellulose binding module (CBM), from transgenic plants or transgenic plant cells expressing said fusion protein, comprising
- (a) disrupting the transgenic plant material;
- (b) adding an extraction liquid to the plant material, thereby creating a mixture of soluble and insoluble plant material, so as to extract the soluble fusion protein from said disrupted plant material to the liquid phase to obtain a protein extract;
- (c) separating the insoluble plant material, comprising cell-wall material and solids, from said protein extract comprising said fusion protein of interest;
- (d) contacting said protein extract to a polysaccharide matrix which binds to said fusion protein;
- (e) washing the matrix with the bound fusion protein with one or more suitable aqueous solutions; and
- (f) eluting the fusion protein from said polysaccharide matrix by adjusting conditions effecting the release of said fusion protein from the matrix,
- thereby obtaining the soluble heterologous fusion protein substantially purified.
2. The method of claim 1 wherein said transgenic plant or plant cell is selected from the group of dicotyledonous plants and monocotyledonous plants.
3. The method of claim 1 wherein said plant cell or transgenic plant is selected from the group of plants including tobacco, rape seed, soy bean, alfalfa, lettuce, barley, maize, wheat, oat and rice.
4. The method of any of claims 1-3, wherein the separation step (c) comprises a method selected from expanded bed adsorption (EBA), precipitation, filtration, centrifugation, or any combination thereof.

5. The method of claim 1 wherein affinity binding to said polysaccharide matrix in step (d) comprises a chromatography step.
6. The method of claim 1, combining steps (c) and (d) in a process step comprising expanded bed adsorption with a polysaccharide matrix, as a measure for simultaneous separation of cell-wall material and solids from said protein extract and affinity binding of said CBM-fusion protein onto the polysaccharide matrix.
7. The method of any of claims 1-6, wherein said conditions effecting the elution of said fusion protein from the matrix are non-denaturing conditions that may be neutral or acidic conditions or involve exposure to carbohydrates, or any combination thereof.
8. The method of any of claims 1-7, wherein said polysaccharide matrix comprises cellulose.
9. The method of claim 8, wherein said cellulose matrix comprises a pharmaceutically compatible cellulose.
10. The method of claim 9, wherein said cellulose is Avicel™.
11. The method of any of claims 1-10, wherein said transgenic plant or plant cell comprises a nucleic acid sequence encoding for a CBM.
12. The method of claim 11, wherein said CBM is heat-stable and remains soluble at elevated temperatures.
13. The method of claim 12, wherein said region coding for a CBM is a region of the xylanase10A gene from *Thermotoga maritima*.
14. The method of claim 13, wherein said region coding for a CBM comprises a sequence depicted as SEQ ID NO: 1, or a sequence encoding the same

amino acid sequence or an amino acid sequence with substantial sequence identity to said sequence.

- 5 15. The method of claim 1, wherein said protein extract is heated to a temperature in the range of 37°C and 100°C, for a period of time in the range of from 1 min to 120 minutes during the process.
- 10 16. The method of claim 16, wherein said heated extract is subjected to the process step comprising expanded bed adsorption with a polysaccharide matrix for the simultaneous separation of solids and affinity binding of said CBM fusion protein from the heated extract.
- 15 17. The method of any of claims 1-16, wherein said heterologous fusion protein comprises a protease.
18. The method of claim 17, wherein said protease is mammalian enterokinase (EK) or an enterokinase active part thereof.
- 20 19. The method of claim 18, wherein said EK comprises a bovine EK catalytic domain (EKc).
20. The method of claim 19, wherein said bovine EKc is encoded by the nucleic acid sequence shown as SEQ ID NO: 2.
- 25 21. The method of claim 1, wherein said fusion protein comprises a CBM and a heterologous polypeptide of interest intercepted by a proteolytic cleavage site.
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